REMARKS

Pending Claims

Claims 1-49 have been restricted. Claims 1-14 and 48-49 have been elected and are pending in this case, subject to rejoinder of eligible claims, as discussed below.

Restriction and Linking Claims

Applicants respectfully disagree with the Examiner's comment that plant claim 1 is not a proper linking claim for product claims 15-41 and 45-47. While appropriate linking claims *include* genus and species or combination and subcombination, properly linked claims also include product and a process/method of making or using the product. See, for example, MPEP 821.04(b) directing rejoinder of processes on allowance of a product claim:

> "However, if applicant elects a claim(s) directed to a product which is subsequently found allowable, withdrawn process claims which depend from or otherwise require all the limitations of an allowable product claim will be considered for rejoinder."

Accordingly, Applicants assert product claim 1 is properly linked to claims that recite a method of using the product of claim 1, as well as to claims drawn to combinations/subcombinations that require the product of claim 1.

35 U.S.C. § 103 - *Rutgersson* with *Kleinhofs:*

Claims 1-6, 9, and 12-13 were rejected by the Examiner as obvious over *Rutgersson et al., 1997,* in view of *Kleinhofs et al., 1978.* Applicants respectfully traverse this rejection.

Rutgersson teaches a method for reducing lipoxygenase activity in barley kernels. In particular, they method comprises soaking kernels in lactic acid and heat treatment of the kernels. In order to reduce lipoxygenase activity significantly, the kernels are heated at a temperature of at least 57°C. Even if the grains can germinate after such a treatment, the treatment merely inactivates lipoxygenase enzymes in a non-inheritable manner. Accordingly, undesirable lipoxygenase activity will be present in a given plant until the lactic acid and heat

treatment is performed. Thus, the method described in *Rutgersson* is not useful for preparing barley plants with a total loss-of-function of LOX-1, as claimed.

Furthermore, *Rutgersson* measure lipoxygenase activity by determining oxygen consumption using an oxygen electrode. In the experimental set-up described in the reference, the conversion of linoleic acid by *any* lipoxygenase is measured, as well as any additional oxygen consumption by other oxygen-consuming enzymes that are present in the barley extract. The enzymatic activities measured in the experiments described in *Rutgersson* thus are the total activities derived from several enzymes with similar substrate profiles. In this respect, it is well known that the barley genome encodes several lipases, several lipoxygenases, and several phytases. Accordingly, the described method is not specific for lipoxygenase activity and in particular, the method does not discriminate between activity of LOX-1 and other lipoxygenases.

Rutgersson does not teach or even suggest the preparation of barley plants carrying a mutation in the LOX-1 gene causing total loss of LOX-1 function. Rather, *Rutgersson* describes a non-specific method for inactivating all types of lipogygenase activity as well as other enzyme activities such as lipase activity, in barley kernels in a non-inheritable manner.

Based on the disclosure of *Rutgersson*, it would not have been obvious for the skilled person to prepare a barley plant carrying a mutation in the LOX-1 gene causing total loss of LOX-1 function. First of all, *Rutgersson* specifically teaches that it is important to minimize lipase activity as well as all lipoxygenase activity, which is achieved by the lactic acid/heat treatment. Furthermore, *Rutgersson* does not teach or suggest a method for preparing a null-LOX-1 barley plant.

Kleinhofs describes a general method for mutagenizing barley using azide, and suggests that the method can be used to produce barley mutants with a desired mutation. Neither *Kleinhofs* nor *Rutgersson* teach or suggest a method for preparing a null-LOX-1 barley plant. Further, neither reference discloses or even hints at a useful method for identifying such barley plants. In particular, the method for determining lipoxygenase activity described in *Rutgersson* is highly non-specific and does not give any information on LOX-1 activity.

Given the lack of specific disclosure in each of the primary and secondary references, Applicants submit it would not have been obvious to the skilled person, based on *Rutgersson* in combination with *Kleinhofs* to produce barley plants having a mutation in the LOX-1 gene causing a total loss of LOX-1 function. Removal of this rejection is requested.

35 U.S.C. § 103 - *Douma* WO02/053721:

Claims 1-14 and 48-49 were rejected as obvious over the PCT publication of *Douma* et al., WO02/053721. Applicants respectfully traverse this rejection.

WO02/053721 nowhere discloses in an enabling manner the generation and isolation of barley plants having a mutation in the LOX-1 gene causing a total loss of LOX-1 function. (null-LOX-1 barley plant). Rather, WO02/053721 describes mutant barley with reduced LOX-1 activity. The barely plants described in WO02/053721 retain at least 10% LOX-1 activity as compared to the wild-type enzyme. See, for example, page 17, lines 18-25 and Figure 13 of the present application.

As described in WO02/053721, not a single null-LOX-1 barley plant was identified after screening as many as 20,000 mutagenized barley plants. Further, the reference describes a mutation frequency of 0.9-2.3 per 10,000 grains was expected, based on a study by Kleinhof *et al.,* 1978, where a mutation frequency of 1 to 2.7 was observed in barley after mutagenesis with azide. Thus, it was expected that in the range of 2 to 5 null-Lox-1 barley plants would be identified after screening 20,000 plants. However, not a single null-LOX-1 mutant was identified. This implies that it is highly unlikely that a null-LOX-1 barley plant can be identified by the methods described.

In contrast, the instant application describes a new and very efficient screening method, which allows a reproducible identification of barley plants with no or very little LOX-1 activity (see, for example, p.34, l. 23-29). According to the present application, kernels or preferably embryos should be used for screening (p.34, l. 27-29; P. 36, l. 16-20). Using these new and efficient screening methods, two different null-LOX-1 mutants were identified by screening less than 15,000 mutagenized barley plants (See Example 1, page 60).

The Examiner notes that barley plants with reduced LOX-1 activity also can be prepared using site directed mutagenesis, chimeric RNA/DNA, or by antisense expression. However, at the time of filing the present application none of the aforementioned methods were described in art in a manner enabling the skilled person to prepare barley plants having a mutation in a particular gene causing a total loss-of function.

In particular, at the time of filing the application it was well known that transgenic plants comprising antisense constructs, in general do express at least some protein. Thus, for example *Robbins et al.,* 1998 (copy attached) describe a number of plants comprising anti-sense DFR. It is apparent that these plants still comprise DFR activity, because products of the DFR pathway (condensed tannins) are detected in all the investigated plants. The authors conclude that the results are comparable to "other examples of heterologous antisense in higher plants" (p. 1142, discussion l. 5-7). *Stahl et al.*, 2004 (copy attached) have described that antisense barley plants may retain as much as 40% of wild type activity.

Accordingly, in the absence of any specific teaching how to achieve an antisense barley plant with a total loss of function of LOX-1, the antisense plants described in WO02/053721 must be considered to express at least some LOX-1 protein. Accordingly, such barley plants have reduced LOX-1 activity, but they are not null-LOX-1 barley plants.

Effective methods for preparing specific mutations using chimeric RNA/DNA or site directed mutagenesis were not developed for use in barley plants at the time of filing the present application. In fact not a single example of successful oligonucleotide-directed gene targeting in barley has been published. In other plants, due to the very low efficiency these methods have only been successfully employed for generation of base changes that result in a phenotype that can be easily selected for, e.g. chemically or visually selectable phenotypes. The very low efficiency of the processes precludes targeting changes for non-selectable traits such a loss-of-function of LOX-1. Sensitive, high-throughput screenings based on chimeric RNA/DNA are currently unavailable.

In a review article from year 2005, *Iida and Terada* (Plant Molecular Biology 59: 205-219)(copy enclosed) list that oligonucleotide-directed gene targeting has been pursued in maize, tobacco and rice – in all cases with the *ALS* gene as a target. It is concluded that it remains to be seen whether this strategy with appropriate modifications can be applicable to genes other than directly selectable genes, such as the *ALS* genes.

Thus, the skilled person, based on the teaching of WO02/053721 would not have been able to prepare barley plants carrying a mutation in the LOX-1 gene causing a total loss of function using chimeric RNA/DNA or site directed mutagenesis.

For at least the reasons discussed above, Applicants assert the claims are not rendered obvious by *Douma* WO02/053721. Removal of this rejection is requested.

Double patenting

The claims of US6,660,915 relate to barley plants carrying a specific mutation in the LOX-1 gene identified by SEQ ID 12 of the patent. Furthermore, the claims of the patent relate to grains of such barley plants, plant products prepared from such barley plants, as well as to methods for producing beer or other beverages using such barley plants.

As is apparent from Figure 13 of US6,660,915, the LOX-1 activity of a barley plant carrying this specific mutation is at least 10% compared to wild type. Accordingly, such barley plants are not comprised within the amended claims of the present application, which are directed to barley plants carrying a mutation in the LOX-1 gene causing a total loss of LOX-1 function.

For at least these reasons, Applicants respectfully request removal of the double patenting rejection.

Conclusion

In light of the forgoing Amendment and Remarks, Applicant's respectfully assert the claims are in condition for allowance. Early notice of such allowance is solicited.

The Examiner is invited to telephone the undersigned attorney for clarification of any of these remarks and/or to otherwise speed prosecution of this case.

Respectfully submitted,

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